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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/069,541	02/27/2002	Tatsuya Haga	31671-176438	1435

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EXAMINER	
STANDLEY, STEVEN H	
ART UNIT	**PAPER NUMBER**
1646	

DATE MAILED: 06/09/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/069,541	HAGA ET AL.	
	Examiner	Art Unit	
	Steven H. Standley	1646	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 January 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *100,102,103,105,107 and 109-112* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) *100,105 and 109* is/are allowed.

6)☐ Claim(s) *102,103, 107 and 110-112* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Response to amendment

1. On Tuesday, May 17th 2005, examiner called Ann S Hobbs, the attorney of record in the instant case and indicated there was allowable subject matter. The examiner suggested changes to claims 107, 109, 110-112. In particular the examiner required cancellation of claim 103, directed to a DNA that hybridizes. Examiner suggested amending claim 107 by crossing out 'wherein,' replacing with 'comprising,' and striking the words "are bound," in the claim. Examiner suggested amending claim 109 by striking the first "the" and replacing it with 'An.' Examiner suggested the word 'thereof' be added in between 'complement' and 'according' in claim 110. Examiner suggested the word 'thereof' be added after 'complement' and before 'according' in claim 111. Examiner suggested striking 'being obtainable' and replacing it with 'obtained' in claim 112. Attorney Ann S. Hobbs was unable to authorize the changes required by the examiner. Accordingly, the examiner has entered a final response as follows in the instant case.

Withdrawn Objections and/or Rejections

2. The rejections to claims 108, 109, and 112 under 35 U.S.C. 101 are withdrawn in view of applicant's amendments of 1/12/05.

3. The rejection of claims 99, 101, 104, 106, 107, 108, and 109 under 35 U.S.C. 112, 1st paragraph, scope of enablement are withdrawn in view of applicant's amendments of 1/12/05.

4. The rejection of claims 99, 101, 104, and 106-109 under 35 U.S.C. 112, 1st paragraph, written description are withdrawn in view of applicant's amendments of 1/12/05.

5. Claim 103 was rejected under 35 U.S.C. 112, 2nd paragraph, as being indefinite. Applicant has amended claim 103 to recite specific hybridization conditions. The rejection is therefore withdrawn.

6. The rejection of claims 99, 104, and 108 under 35 U.S.C. 102(b) for reasons made of record in the office action of 10/24/04 are withdrawn, as applicant has cancelled the claims.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. The rejection of claim 103 is maintained for the reasons made of record and the reasons stated below.

It is further noted that applicant has added specific stringency conditions into claim 103 in response to the (prior) examiner's suggestions. However, this is not found to be persuasive.

The examiner has calculated (using both the equations provided on page 4 of Appendix A, and roughly 1 percent mismatch resulting in 1 degree centigrade loss in melting temperature, or Tm, which is also described in Appendix A) that when using a full-length molecule according to SEQ ID NO: 5 of claim 102 (as recited in claim 103), the full-length probe would have a Tm of 42°C when hybridizing to a nucleic acid with only about 63 percent sequence identity to that of SEQ ID NO: 5.

The specification fails to provide guidance for the scope of variants, fragments, polymorphisms, isoforms or analogs that hybridize to SEQ ID NO: 5 (which encodes a high affinity choline transporter) under the recited stringency conditions. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with Claim 103 for these reasons and the reasons set forth at pages 3-8 of the office action dated 5/26/04.

8. The rejection of claim 103 under 35 U.S.C. 112, 1st paragraph, written description is maintained for reasons made of record in the office action of 10/24/04, and for the reasons given below.

Claim 103 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement for the reasons made of record on in the action of 10/24/04 and below. Applicant has amended claim 103 to recite specific stringency conditions. Applicants changes have been fully considered and found not to be persuasive. The claim contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification does not teach the (splice) variants, fragments, polymormisms, isoforms and analogs of the nucleic acid sequence of SEQ ID NO: 5 under the hybridization conditions recited.

One skilled in the art would ***not*** know various genomic splice-variants encoding functionally unique forms of the claimed polypeptide encoded by the nucleic acid of SEQ ID NO: 5. For instance, Modrek et al. (2002) report that 40-60% of all human proteins are alternatively spliced (abstract, line 1-2). Alternative splicing adds or takes away nucleic acid to alter the nucleic acid and thereby nearly always altering the encoded polypeptide. Further, 70-88% of all splice changes alter the protein product (thus, also altering the nucleic acid sequence; page 14, left column, 2nd paragraph). Further yet, most of the changes are "functionally interesting [page 14, left column, 2nd paragraph]," indicating that the changes confer differences that contribute to many aspects of a proteins function. Thus, these splice variations are of high probability to

occur, modify function, and are not taught in the specification and are unknown and undiscovered.

Therefore, only the isolated nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 5, but not the full breadth of the claim meets the written description provision of 35 U.S.C. 112, 1st paragraph.

New Claim Objections

Claims 110 and 111 are objected to because of the following informalities: Claim 110 does not refer to what is a complement of the DNA. It is suffested the applicant add the word 'thereof' after 'complement' and before 'according.' Appropriate correction is required.

Claim 111 is objected to for the same reasons that claim 110 is objected to. It is suffested the applicant add the word 'thereof' after 'complement' and before 'according.' Appropriate correction is required.

New Rejections Under 35 U.S.C. 112, 2nd Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 102, 103, 107, and 110-112 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 102 is rejected for being indefinite because the claim recites "human derived," and it is unclear in what way this limits the claim. The sequence can be construed as coming from an animal other than human since it is only "human derived." That is, a human may have derived it from another animal. Claims 103, 110-111 are rejected as being dependent on claim 102.

Claim 107 is rejected for being indefinite because the claim recites "wherein....a marker protein and/or peptide are bound." This is indefinite because one does not clearly know whether "a marker" and protein of SEQ ID NO: 6 are bound together.

Claim 112 is rejected for being indefinite because the claim recites "being obtainable." This is indefinite because it suggests the choline transporter of claim 112 is obtainable in other (indefinite) ways.

Conclusion

Claims 100,105, [redacted] and 109 are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Steven H. Standley whose telephone number is (571) 272-3432. The examiner can normally be reached on 8:00-4:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Anthony Caputa can be reached on (571) 272-0829. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Steven Standley, Ph.D.

Lorraine Spector, Primary Examiner

Appendix A

Molecular Biology Techniques Manual

Third Edition

Edited by:

Vernon E Coyne, M Diane James, Sharon J Reid and Edward P Rybicki

DETECTION OF NUCLEIC ACIDS BY HYBRIDISATION

Contents

INTRODUCTION

Hybridisation is a term used to describe the specific complementary association due to hydrogen bonding, under experimental conditions, of single-stranded nucleic acids. It should more properly be referred to as "annealing", as this is the physical process responsible for the association: two complementary sequences will form hydrogen bonds between their complementary bases (G to C, and A to T or U) and form a stable double-stranded, anti-parallel "hybrid" helical molecule. One may make ones nucleic acid single-stranded for the purpose of annealing - if it is not single-stranded already, like most RNA viruses - by heating it in 0.01M NaCl to a point above the "melting temperature" of the double- or partially-double-stranded form, and then flash-cooling to ±0oC: this ensures the "denatured" or separated strands do not re-anneal.

Alternatively, one may denature DNA reversibly by treatment with 0.5M NaOH: this does not work for

RNA, as this hydrolyses under these conditions.

Why would one want to anneal pieces of nucleic acid?

The answer is simple: nucleic acid hybridisation on membrane filters is a simple, sensitive, and specific means of detecting nucleic acid sequences of interest. One immobilises "target" nucleic acid - denatured so as to be effectively single-stranded - on an absorptive, porous membrane, and then anneals to it an appropriately "tagged" or "labelled" single-stranded probe nucleic acid. After washing off unannealed probe, one detects the immobilised hybrid by means of the label: this is often 32P incorporated into a nucleotide, which allows autoradiographic or scintillometric detection.

One may also use non-radioactive labels and detection systems, for sensitivities of detection down to picogram levels. The system of choice at the moment appears to be the Boehringer Mannheim DIG (digoxigenin) non-radioactive labelling and detection kit, which uses digoxigenin-11-dUTP as a substituted nucleotide which is enzymatically incorporated into DNA.

The mechanism of immobilisation of nucleic acids on membranes is not fully understood: nitrocellulose strongly binds only ss-nucleic acids (ssNA), under conditions of high salt (>1M NaCl), and has to be heated at 80oC in a vacuum to irreversibly attach the NA; nylon membranes (Hybond-N, GeneScreen) bind all nucleic acids under a wide range of salt concentrations, and irreversible or covalent attachment can be achieved by UV irradiation for 5 min or less, or by treatment with 0.4M NaOH.

The complementary association of two strands of polynucleotides

is the basis for replication of all organisms; the complexity inherent in the sequence of the molecules renders the association extremely specific for any molecule longer than sixteen nucleotides. This is easily understood if one considers the combinatorial possibilities of given lengths of "probe" sequence: there is a ¼ chance (4-1) of finding an A, G, C or T (U for RNA) in any given DNA sequence; there is a 1/16 chance (4-2) of finding any dinucleotide sequence (eg. AG); a 1/256 chance of finding a given 4-base sequence. Thus, a sixteen base sequence will statistically be present only once in every

416 bases (=4 294 967 296, or 4 billion):

this is about the size of the human genome, and 1000x greater than the genome size of *E. coli*.

Thus, the association of two nucleic acid molecules - presumed to be at least a few hundred bases long - is an extremely sequence-specific process, far more so than the widely-used specificity of monoclonal antibodies in binding to specific antigenic determinants. The correct annealing of two sequences to each other does, however, depend on the physical and chemical solution conditions under which the reaction takes place.

Melting Temperatures

For example, all double-stranded nucleic acids - whether dsDNA, dsRNA or RNA:DNA hybrids - have specific "melting temperatures", which depend mainly upon their specific guanine+cytosine content, but also upon whether they are DNA, RNA, or a mixture (RNA:RNA hybrids have the highest melting temperatures, followed by DNA:RNA hybrids, then dsDNA), and upon the ionic strength of solution.

The melting temperature is also dependent upon the length of the sequences to be annealed: the shorter the probe sequence, the lower the melting temperature. The degree of sequence mismatch also determines the effective melting temperature of a hybrid: Tm decreases by about 1oC for every 1% of mismatched base pairs. It therefore makes sense to maximise probe length in order to minimise Tm reduction due both to length and degree of sequence mismatch. Under standard conditions of annealing (0.8M NaCl, neutral pH) one may calculate the **melting temperature** ™ of any given DNA hybrid as shown:

Tm = 81.5oC + 0.41(%G + %C) - 550/n

where n=probe length (no. nucleotides).

One can see that the reduction in Tm becomes negligible for probes of length 200 nt or greater. Thus, one may vary the specificity of association of a specific single-stranded "probe" and a target by varying the incubation temperature of the annealing reaction: the higher the temperature, the higher the specificity of the reaction - and the lower the likelihood of annealing taking place.

Hybridisation Stringency

The successful use of nucleic acids as probes for sequences of interest therefore depends upon certain reaction conditions which are in turn determined by the physical properties (ie. length and sequence) of the probe. This leads to the concept of ***stringency*** of hybridisation: one increases the stringency by lessening the likelihood of non-homologous annealing. This can be done by simply increasing the temperature of incubation - bearing in mind that *rate* of hybridisation/annealing is maximal at about Tm - 25oC, and too high a temperature results in very slow annealing. An acceptable compromise is to anneal at a standard temperature (eg. 65oC), and then *wash* the annealed and immobilised hybrid molecules to varying degrees of stringency: the extent to which one should wash can be assessed by repeated autoradiography, if the probe is 32P-labelled, or by repeated colour assay of replicates in the case of non-radioactively labelled probe. Washing stringency may be increased by varying the ionic strength (from 1.0M NaCl to 0.02M), or varying the temperature (ambient to 65oC). One may also include SDS or other detergent in wash and in hybridisation buffers in order to decrease non-specific attachment of probe to the adsorptive membrane. For this reason a *blocking or prehybridisation buffer* is normally used before and during the annealing reaction, to block adsorptive sites on the membrane not occupied by target nucleic acid. This normally consists of buffer salts, detergent, protein, inert polymer material, and DNA.

It is possible to include various other constituents in annealing buffers, designed to increase the hybridisation rate, or the stringency, or both. *Formamide* is a helix destabiliser, and enables one to decrease annealing temperature: the presence of **formamide** decreases the Tm as shown:

TFm = Tm - 0.61(%formamide, w/v)

It is most often used in annealing reactions using RNA as target or probe, and especially with dsRNA hybrids, as these have high Tms which necessitate elevated reaction temperatures. Standard conditions using formamide would be 42oC with 50% formamide content in the annealing buffer. Formamide also decreases the rate of annealing, so one normally includes substances like dextran sulphate - a polyanionic polymer - as "molecular exclusion agents" to decrease the volume of solvent available to the probe. Polyethylene glycol is a far cheaper and equally effective substitute for increasing reaction rate. Too high a concentration of DS or PEG raises "background" or non-specific probe attachment to unacceptably high levels. Their effectiveness is also directly proportional to probe length, and they are useless when oligonucleotides of less than 50 nt in length are used as probes.

Summary

A standard hybridisation reaction, then, consists of ***probing*** an immobilised ***target sequence*** on a membrane with a ***labelled specific probe sequence***: this is done by annealing the probe to the target under (usually) standard "hybridisation conditions" of 0.9M NaCl, 65oC, for 4-16 hr. Probes are usually molecules of DNA or cDNA, a few hundred nt to several kilobases long, cloned into and grown up as recombinant plasmids in *E. coli*, and purified by caesium chloride gradient centrifugation. One may also use nucleic acid directly purified from the organism of interest, but this is only really effective if this is a virus or a plasmid, as otherwise the probe length is too great, and the repeat number is too small to give appreciable signal. In other words, probes should not be too long, as otherwise one needs very high concentrations of nucleic acid in order to guarantee a sufficient number of copies of the sequence in order to give a detectable "signal" for detection purposes.

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